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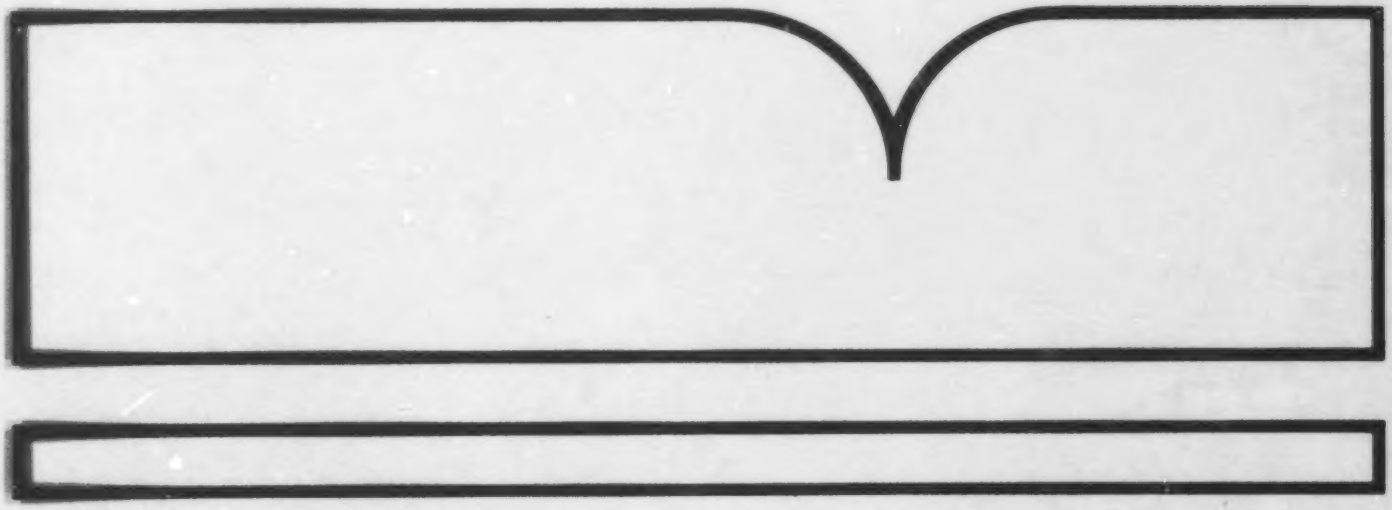

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ANALYSIS OF THE SPECTRA OF GENETIC ACTIVITY IN SHORT-TERM TESTS

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16. ABSTRACT

For agents classified by IARC as known or suspected human carcinogens and for over 100 other chemical pesticides, solvents, intermediates and final products, we have extracted and recorded qualitative and quantitative genetic bioassay data available in the literature. Either the lowest effective dose (LED) or the highest ineffective dose (HID) tested was recorded for each agent and bioassay system. The systems were organized according to classes of genetic activity and subdivided by the phylogenetic level of the test organism. For each compound, the quantitative results in the test systems were represented by computer-generated bar graphs, so-called "genetic activity spectra." Statistical methods and pattern recognition techniques were used to evaluate the activity profiles of compounds in a pairwise manner. The results showed that structurally similar compounds frequently displayed qualitatively and quantitatively similar profiles of genetic activity. As the database is enlarged, the analysis of genetic activity profiles may contribute to our understanding of structure-activity relationships and mechanisms of action of chemical agents. Such a method of presentation could well serve as a medium for international exchange and evaluation of genetic bioassay data.

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ANALYSIS OF THE SPECTRA OF GENETIC ACTIVITY IN SHORT-TERM TESTS

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INTRODUCTION

We report here our efforts to organize data resulting from tests for genotoxicity of chemical compounds and to display it for evaluation through the use of computer generated bar graphs called genetic activity spectra or profiles. These graphs reflect the lowest effective doses (LED) or highest ineffective doses (HID) resulting from tests reported in the open literature (or in publicly available contract final reports). Thus, quantitative results from up to 100 different test systems may be represented for each compound and each LED or HID reported for each test system may be plotted automatically. These individual LED or HID values may be marked to indicate the influence of an exogenous metabolic activation system. The genetic activity profile of acrylonitrile is shown in Fig. 1 as an example.

METHODS

In practice, the doses or concentration terms for all *in vitro* tests are converted to µg/ml and those for *in vivo* tests are expressed as mg/kg. A scale illustrating the log dose units (LDU) plotted on the y-axis is shown in Fig. 2. The sequence of test systems currently plotted on the x-axis is shown in Table 1 together with the corresponding three-letter test system code words. The sequence is largely phylogenetic within the major categories of genetic damage: DNA damage, (gene) mutation, chromosomal effects, cell transformation, and effects on sperm.

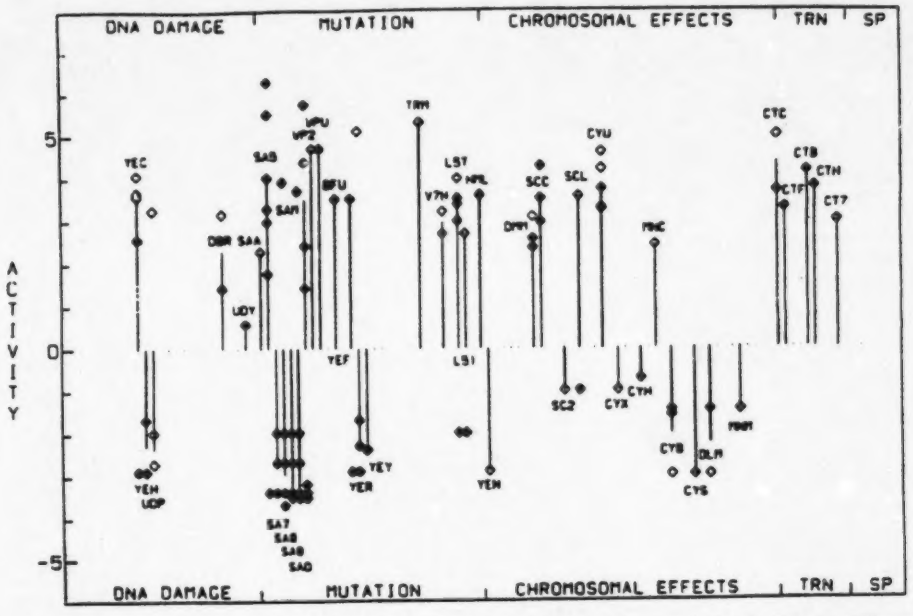

Figure 1. Genetic activity profile for Acrylonitrile. Symbols indicate LED or HID values, ◇ without or ◆ with exogenous activation.

In genetic activity profiles published or presented by us to date (Waters et al., 1984, Garrett et al., 1984, Vainio et al., in press; Waters et al., in press), the result for a given test system was scored positive or negative according to the majority of the data; and the appropriate subset of the data was used to plot the average of the logarithmically transformed LED or HID values. In the few cases where the numbers of positive and negative results were equal, we considered the overall result to be positive. It is certainly possible to reflect a committee evaluation in the plotting of the data instead of the mechanical procedure just described. In fact, such a committee evaluation was followed in the derivation and publication of genetic activity profiles in the appendix to a forthcoming IARC monograph (1985).

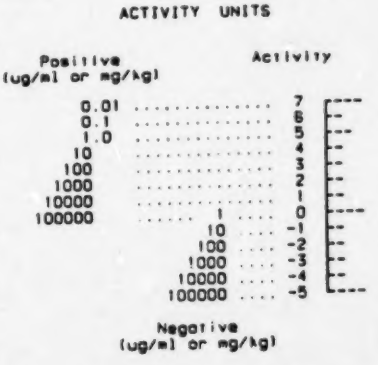

Figure 2. Units shown on the x-axis of a genetic activity profile.

TABLE 1. Sequence of Codes Assigned to Individual Test Systems

DNA damage

Prokaryotes

Escherichia coli Pol A (W3110-P3478)
- SID Spot test
- SET Liquid suspension

Bacillus subtilis rec (H17-M45), spot test
- REC DNA repair-deficient bacteria

Fungi

Saccharomyces cerevisiae
- YEC Gene conversion
- YEH Homozygosis (through recombination or gene conversion)

Mammalian cells (in vitro)

Unscheduled DNA synthesis
- UDP Rat primary hepatocytes
- UDH Human diploid fibroblasts
- UDL HeLa cells
- UDS Other mammalian cells
- REO DNA repair, other than UDS

Inhibition of DNA synthesis
- IDH Human cells, normal
- IDL HeLa cells
- IDR Rodent cells

DNA strand break
- (DBH) Human cells
- (DBR) Rodent cells

Mammals (in vivo)

Unscheduled DNA synthesis,
- UDM Mice
- UDR Rats

Humans (in vivo)
- UDY UDS in lymphocytes

Mutation

Prokaryotes/Bacteriophages

Salmonella typhimurium
- SAA Forward Mutation
- Reverse Mutation
  - SA5 TA1535
  - SA7 TA1537
  - SA8 TA1538
  - SA9 TA98
  - SA0 TA100
  - (SAM) Miscellaneous strains

E. coli
- WP2 WP2
- WPU WP2 uvrA
- (ECO) Miscellaneous strains
- (BFU) Body fluids, urine
- HMA Host mediated assays

Fungi/green plants

Saccharomyces carevisiae
- YEF Forward mutation
- YER Reverse mutation
- YEY *Schizosaccharomyces pombe*, fwd. mut.

Aspergillus
- ASF Forward mutation
- ASR Reverse mutation

Neurospora crassa
- NEF Forward mutation
- NER Reverse mutation
- ARM Arabidopsis mutation
- HOM *Hordeum vulgare*
- TRM Tradescantia mutation

Insects
- SRL *Drosophila melanogaster*, sex-linked recessive lethal test

Mammalian cells (in vitro)

Chinese hamster cells
- CHO Ovary HGPRT or ATPase locus
- V7H Lung (V79) HGPRT locus
- V70 Lung (V79) ATPase locus

Mouse lymphoma (L5178Y cells)
- L5T TK locus
- L5I Other loci

Mammals (in vivo)
- MST Mouse spot test

Chromosomal effects

Prokaryotes/Fungi
- HML Host mediated assay
- YEN *Saccharomyces cerevisiae*, aneuploidy

Plants
- ALC *Allium cepa*, cytogenetics
- VIC *Vicia faba*, cytogenetics
- PYC Other plant chromosome studies

Insects

D. melanogaster
- DAN Aneuploidy-all tests
- DHT Heritable (reciprocal) translocation
- (DMM) Mosaics

Mammalian cells (in vitro)

Sister chromatid exchanges
- SCC Chinese hamster ovary (CHO)
- SCO Chinese hamster DON cells
- SCV Chinese hamster V79 fibroblasts
- SC2 Other mammalian cells excluding human
- SCF Human fibroblasts
- SCL Human lymphocytes
- SCM Human lymphoblastoid cells
- SC1 Other human cells

Chromosomal aberrations
- CYU Chinese hamster
- CYV Syrian golden hamster
- CYX Rat
- CYY Mouse
- (CYC) Other non-human cells
- CYH Human lymphocytes
- (CYZ) Other human cells
- MNC Micronuclei in vitro

Mammals excluding humans (in vivo)
- SC3 Sister chromatid exchanges

Chromosomal aberrations
- CYB Bone marrow
- CYL Lymphocytes or leukocytes
- CYO Oocyte or early embryo studies
- CYS Spermatogonia treated and observed
- CYT Spermatogonia treated, spermatocytes observed
- DLH Dominant lethal test, mouse
- DLR Dominant lethal test, rat
- MHT Heritable translocation, mouse
- MNN Micronucleus test, hamster
- MNM Micronucleus test, mouse
- MNR Micronucleus test, rat

Humans (in vivo)
- SC4 Sister chromatid exchanges
- CYD Chromosomal aberrations, lymphocytes

Cell transformation

Syrian hamster embryo
- CTC Clonal assay
- CTF Focus assay
- CTS Transformation strains
- CTA AKR/ME cells
- CTB BALB/c3T3 cells
- CTH C3H10T1/2 cells
- CTK BHK21 cells
- CTR RLV/Fischer rat embryo cells
- CT7 SA-7 virus/SHE cells

Effects on sperm
- SPH Human
- SPI Mouse
- SPF Mouse F_1 assay

A computer program was written to compare the genetic activity profiles of any pair of chemical compounds. Where common test systems are utilized to test compounds, agreement between the test results can be determined by examining the positive or negative direction and magnitudes of responses. The comparison is made by first calculating the difference in the values of the logarithmic responses,

$$|LDU_{ija} - LDU_{ijb}| \qquad (1)$$

where j_a and j_b denote the two chemicals being compared, and i the test system. A dose-related function (DRF) is a measure of agreement in the magnitudes of lines of the genetic activity profiles. The DRF is defined from a sum of the LDU values derived from the common genetic tests:

$$DRF = \frac{1}{n} \sum_{i} c - |LDU_{ija} - LDU_{ijb}| \qquad (2)$$

where n is the total number of common tests, and c is a constant. The constant c is arbitrarily assigned a value of 3.0. A DRF value of 2.0 corresponds to an average dose difference of one logarithmic unit. Fig. 3 illustrates the mechanics of comparing test spectra of two chemicals for common tests and dose-related features. By the same procedure, a second dose-related function (DRF_c) was determined solely from the concordant test results.

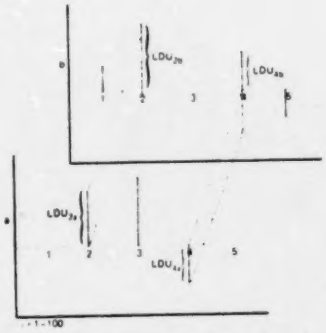

Figure 3. Schematic of the genetic activity spectra showing 5 bioassays of 2 chemicals. j_a (abbreviated a) and j_b (abbreviated b). Bioassays 2 and 4 are common to both chemicals; however, conflicting results for test 4 cause the value of DRF to be substantially reduced, agreeing with the observation of disparity in the genetic activity spectra.

Further details on the methodology for representation and analysis of genetic profiles may be found in Garrett et al. (1984).

RESULTS

To date we have entered about 4,000 data units for approximately 200 test systems applied to 130 chemicals. A data unit consists of the test system code, a positive or negative result shown without and with exogenous metabolic activation, the LED or HID, reference number(s), and an abbreviated citation. Much of this information (60 percent) describes 29 known or suspected human carcinogens. These compounds are listed as classified by IARC (1982) in Table 2. We have reviewed the clinical and epidemiological data and have described the qualitative genetic bioassay data for 24 of these compounds (Waters et al., 1983). Quantitative information has been added and a comparative computer analysis (cf. Fig. 2) of the activity profiles of these 24 agents has been reported (Garrett et al., 1984). By selecting the significant relationships between chemical pairs ($P<0.1$), four groups of compounds were identified in the initial computer analysis.

Group I was composed of the primary aromatic amines (2-naphthylamine, 4-aminobiphenyl and benzidine) and acrylonitrile. Group II was composed of two nitrogen mustards (cyclophosphamide and melphalan), an epoxide (ethylene oxide), and a reactive substituted alkene (vinyl chloride). A link between Groups I and II, designated Group I-II, was formed by 3 compounds (aflatoxin B_1, epichlorohydrin and chromium compounds) whose profiles matched the profiles of members of both Groups I and II. Group III consisted of arsenic, cadmium, auramine and diethylstilbestrol.

As a result of our continual updating of the data base on these agents, we have refined the comparative computer assessment of these compounds. Also we have extended the data base to include other compounds representing pesticides, intermediates, solvents, etc. Here we describe the comparative assessment of the genetic activity profiles of the known or suspected human carcinogens.

From the computer-generated genetic activity spectra for the 29 chemicals studied, 406 pairwise combinations were obtained. To identify compounds that displayed similar genetic activity profiles, the data were examined (1) for all common bioassays conducted for each pair of chemicals and (2) for qualitative and quantitative agreement of individual profile lines. No statistically significant correlation was found in

TABLE 2. IARC Classification (1982) of Agents in the Present Data Base

Group	Classification	Agents
1	Causally associated with cancer in humans	4-Aminobiphenyl Arsenic and arsenic compounds[a] Asbestos Azathioprine Benzene Benzidine N,N-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine) Bis(chloromethyl) ether and technical-grade chloromethyl methyl ether Chlorambucil 1,4-Butanediol dimethanesulfonate (Myleran) Chromium and certain chromium compounds[a] Cyclophosphamide Diethylstilbestrol Melphalan Mustard gas 2-Naphthylamine Vinyl chloride
2A	Higher degree of evidence for probable human carcinogenicity compounds	Acrylonitrile Aflatoxins Nickel and certain nickel compounds[a] Phenacetin
2B	Lower degree of evidence for probable human carcinogenicity	Auramine Cadmium and certain cadmium compounds[a] Chloramphenicol Ethylene oxide Epichlorohydrin Phenytoin

[a]The specific compounds which may be responsible for the carcinogenic effect in humans cannot be specified precisely.

the profiles for 12 of the compounds: auramine, benzene, BCME, chlorambucil, chloramphenicol, chlornaphazine, CMME, DES, mustard gas, myleran, phenacetin, or phenytoin. However, of the 406 possible pairings, only 26 pairings showed agreement greater than that which could be expected by chance: the probability (P) that the number of congruent results would occur by chance is given by the relation:

$$P = \sum_{i=n-m}^{n} \binom{n}{i} (0.5)^n \qquad (3)$$

where 0.5 is the probability of the results matching for any common tests, n is the number of common tests, m is the number of conflicting tests, and $\binom{n}{i}$ are the binomial coefficients defined as $n!/[(n-i)!i!]$, $n \geq i \geq 0$.

The matching process for the known or suspected human carcinogens was performed in two stages. The first stage considered all matches of $P \leq 0.1$. The second stage considered only matches at $P \leq 0.01$. The highly significant ($P \leq 0.01$) qualitative matches between pairs of chemicals with more than 10 tests in common are shown in Table 3. Also included for comparative purposes are two additional matches with slightly higher P values (see Table 3). In the analysis published previously (Garrett et al., 1984), 4 groups of chemicals were segregated from the possible pairings; however, only 2 truly distinct groupings were present since members of Groups I and II were linked with members of Group I-II. In the current analysis this combined group is designated as Group 1, and the former Group III is redefined as Group 2.

Nine of the eleven compounds matched in Group 1 with a probability $P \leq 0.1$ were again related at a higher significance level ($P \leq 0.01$). Acrylonitrile and vinyl chloride failed to match with the Group 1 compounds at the selected significance level. Acrylonitrile now matches with compounds in Group 2 including members in the prior analysis, cadmium and arsenic [+3] compounds. Group 2 also contains nickel, arsenic [+5] and asbestos compounds as can be seen in Table 3. At a lower level of significance ($P \leq 0.02$), vinyl chloride also matches with acrylonitrile.

TABLE 3: Highly Significant Matches for Known/Suspected Human Carcinogens

Group 1	N[a]	M[b]	P[c]	DRF	DRFc
2-Naphthylamine					
4-Aminobiphenyl	21	4	.004	1.35	2.31
Aflatoxin B_1	28	5	.000	.64	1.53
Benzidine	34	8	.001	1.02	2.24
Chromium Compounds[+6]	24	5	.003	.95	2.05
Cyclophosphamide	32	9	.010	.64	2.08
Epichlorohydrin	29	3	.000	1.68	2.12
Aflatoxin B_1					
4-Aminobiphenyl	16	3	.011[d]	.55	1.39
Benzidine	31	6	.000	.11	1.26
Chromium Compounds[+6]	32	3	.000	1.59	1.91
Cyclophosphamide	43	8	.000	.47	1.40
Epichlorohydrin	28	3	.000	.77	1.17
Chromium Compounds[+6]					
4-Aminobiphenyl	15	2	.004	1.28	2.07
Benzidine	24	5	.003	.67	1.72
Cyclophosphamide	40	11	.003	.41	1.46
Epichlorohydrin	24	4	.001	1.03	1.70
Cyclophosphamide					
Benzidine	32	9	.010	.77	2.13
Epichlorohydrin	36	10	.006	.77	1.97
Ethylene oxide	24	1	.000	1.59	2.01
Melphalan	18	0	.000	1.87	1.87
Epichlorohydrin					
Ethylene oxide	18	4	.015[d]	.40	1.40
Group 2					
Acrylonitrile					
Cadmium Compounds	14	2	.006	1.01	1.68
Nickel Compounds	16	2	.002	1.47	2.01
Arsenic Compounds[+3]					
Acrylonitrile	17	3	.006	.85	1.86
Cadmium Compounds	11	1	.006	2.20	2.51
Asbestos					
Arsenic Compounds[+5]	11	1	.006	1.85	2.30
Nickel Compounds	11	1	.006	1.45	1.84

[a]N = number tests in common; [b]M = discordant results;
[c]P = probability according to equation (3);
[d] = data showing P>0.01 included for comparative purposes.

Cyclophosphamide is the most tested compound in the data base having been studied in 75% of the tests shown in Table 1. Cyclophosphamide matched with more compounds (7) in the comparative analysis than did any other compound. However, diethylstilbestrol was tested across 55 different assays in the matrix and this compound failed to match any other compound at the significance level ($P \leq 0.01$) selected. This indicates that the utilization of various test systems will improve the chances of matching compounds only when the compounds cause similar genetic effects.

A quantitative comparison (Table 3) of common bioassays is given by the dose related function (DRF). A value ≥ 2 indicates that the effective or ineffective doses for common bioassays agree within an order of magnitude, or one logarithmic unit. Most of the values for the DRF were less than 2, indicating less quantitative agreement in the responses of the common test systems than reported initially (Garrett, et al., 1984) with fewer data. The index of dose as a function of the concordant tests (DRF_C) also was investigated: this function averaged 1.85 with a standard deviation of 0.35 for all values listed in Table 3. Many of the values for DRFc were approximately 2, indicating substantial quantitative agreement in the responses of the common concordant test systems.

With new data added, the probability remained highly significant that 2-naphthylamine matches qualitatively with benzidine, 4-aminobiphenyl and epichlorohydrin in Group 1. For concordant tests on these chemicals, the dose related quantitative matches were within one order of magnitude on the average. 2-naphthylamine also displayed highly significant matches with aflatoxin B_1, chromium [+6], and cyclophosphamide. Similar results were obtained with matches between cyclophosphamide and 6 other compounds as can be seen in Table 3. Aflatoxin B_1, chromium [+6], and epichlorohydrin (formerly constituting group I-II) match with Group 1 chemicals, but as in the previous analysis the quantitative matches are not impressive.

Nickel did not show any matches with the other compounds in the initial investigation; however, in the present report nickel displays matches at highly significant levels of probability with the other new members of Group 2, acrylonitrile and asbestos. These matches exhibit close quantitative agreement for concordant tests. The best quantitative match among Group 2 compounds linked trivalent arsenic compounds with cadmium.

DISCUSSION

In a prior analysis (Garrett et al., 1984) and again in the current evaluation of compounds considered as known or suspected human carcinogens, we discovered two distinct groupings of compounds. Examination of the activity profiles of the specific agents revealed that Group 1 compounds produced primarily positive results in all categories of genetic damage. Seventy-four percent of the 140 mutation assays used with Group 1 compounds gave positive results. More than 90% of the assays used to measure either DNA damage (73/79) or chromosomal effects (112/123) produced positive results, and all 33 cell transformation assays were positive with Group 1 compounds. The only assay used in screening these compounds that consistantly gave negative results (4 of 5 compounds tested) was the sperm abnormality test using mice (SPI). Agents in Group 2 displayed both positive (90) and negative (70) test results. The Group 2 compounds produced positive results in 16 of 17 cell transformation assays and in 30 of 41 assays for chromosomal effects using mammalian cells *in vitro*. They frequently displayed negative results in the Salmonella tester strains (23 of 26) and in chromosomal effects assays using mammals *in vivo* (15 of 20).

Compounds not matching with Group 1 or Group 2: (1) were tested in very few assays - e.g., chlorambucil, chlornaphazine, BCME, CMME, myleran and mustard gas, (2) gave numerous negative results - e.g. auramine, benzene, chloramphenicol, chromium [+3] compounds and DES, or (3) produced a combination of the two conditions - e.g. phenacetin and phenytoin. The fact that some of these compounds yielded negative results for some tests within a given genetic endpoint should not be overly emphasized. In fact, benzene, auramine, chromium [+3] and DES produced positive responses for each of the genetic endpoints studied.

The comparative analysis described above also facilitated a quantitative assessment based on the lowest effective or highest ineffective dose levels. For the compounds selected on a qualitative basis and shown in Table 3, the mean dose related function for concordant assays DRFc was 1.9 which indicates that the doses agree within 1.1 logarithmic unit. The combined methodology permits rapid comparative evaluation of genetic toxicological data and may be useful in directing further genetic and mechanistic studies.

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